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ON THE PROPERTIES AND TECHNIQUE OF OBTAINING THE STREPTOCOCCUS AEROSOL IN EXPERIMENTAL CONDITIONS

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ON THE PROPERTIES AND TECHNIQUE OF OBTAINING THE STREPTOCOCCUS AEROSOL IN EXPERIMENTAL CONDITIONS

[Following is the translation of an article by Ye. P. Sinelnikova, Kievskiy Institute for the Advancement of Doctors, published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology Epidemiology and Immunobiology) #1, 1965, pages 120-123. The article was submitted to the editors on 22 January 1964. Translation performed by Sp/4 Richard M. Koplen]

The mission of the work was the study of a model of a streptococcus aerosol and the explanation of the influence on its biological stability of humidity, temperature, and the composition of the dispersing liquid. Until recently, the model of streptococcus aerosol was used very limitedly in the study of the pathogenesis of streptococcus aeroinfection. The reason was the difficulties connected with biological instability -- the rapid loss of viability by the microorganism which is found in the aerosol state (Sonkia, 1950, 1951; Coburn, 1951, 1954, 1957).

Data available in literature concerning the influence of humidity on the viability of streptococci in an aerosol are negligible and extremely contradictory. Wells and Zappasodi (1942) asserted that hemolytic streptococci die rapidly in a dry atmosphere and are well preserved in a damp one. According to data of Dunklin and Puck (1948), the most intensive dying off of microorganisms occurred at a relative humidity near 50%; at lower and higher indices the streptococci survived longer. Based on Webb's data (1959, 1960), the mechanism of microbe loss for various indices of relative humidity depends on the composition of the dispersed liquid. He succeeded in explaining the influence of the protein component in a droplet on the survival of the microbial cell.

In the present report, investigations are introduced on the reproduction of an experimental model of a streptococcus aerosol in a hermetic aerosol chamber made of organic glass with a 40 liter capacity.

For reproduction of the bacterial aerosol, 2 strains of β-hemolytic streptococcus were used: St. pyogenes No. 654 and

St. pyogenes Dochez. In the test, an 18-hour culture of streptococcus in 0.25% glucose meat-peptone broth was used. The culture was centrifuged; from the precipitate a suspension was prepared based on the optical bacterial standard in various liquids: 1) 0.85% physiological solution of NaCl; 2) Ringer-Locke's solution with 0.1% gelatin (Coburn, 1951); 3) meat peptone broth; 4) 1% solution of blood serum (Sinelnikova, 1961). The suspensions were kept in a refrigerator and used over a period of a few days. Dispersion of the bacterial suspension in the chamber was brought about through a disperser of the "Chicago" type, under pressure caused by the speed of air movement, equal to 25 l./min. A monodispersed aerosol was produced in the chamber. The size of the droplets ranged from 5--10 r k. The measurement of the size of the droplet and the study of the degree of aerosol dispersion was accomplished based on the method described by Yelkin and Eydelshteyn (1955). The bacterial suspension was prepared in a Ringer-Locke's solution with 1% gelatin.

For removal of aerosol specimens, we used liquid effervescent filters which were prepared from meat-peptone broth (Sinelnikova, 1963) and the Rechmenskiy bacteria trap (Rechmenskiy, 1952). One ml. meat-peptone broth was placed in a sterile device and, after sorting the specimens, broth from the device was seeded in a petri dish with 5% blood nutrient agar. After an 18-hour incubation, the growth of the colony of β-hemolytic streptococcus was considered, their number calculated, and a recalculation was made for 1 liter of aerosol.

The concentration of viable cells of hemolytic streptococcus in the aerosol was studied during dispersion of 0.3 -- 0.5 ml. of a bacterial suspension, containing 5.10^8 or 1.10^9 cells in 1 ml. The test lasted 2 hours and 30 minutes at 18^o and the relative humidity was 58--62%. Removal of the specimens was conducted after 15 minutes.

During dispersion of 0.3 ml. of bacterial suspension with a concentration of viable cells equal to 6.15 -- 10^7 in 1 ml. on an average, it was possible to obtain a comparatively stable aerosol of hemolytic streptococcus. Over a period of 15 minutes from the moment of dispersion, the concentration of viable cells in the aerosol was reduced 5--$2^1/2$ times, after which the aerosol was partially stabilized and preserved biological activity for 2 hours. During the dispersion of smaller volumes of the bacterial suspension, it was possible to obtain a streptococcus aerosol which was preserved up to 45 min.

In the second series of tests, we studied the indices of survival of β-hemolytic streptococcus in an experimental aerosol in relation to the compostion of the liquid of the bacterial suspension. In the chamber we dispersed 0.3 ml. of bacterial suspension, containing 1.10^9 cells in 1 ml. Observations continued for 2 hours at 20-21° and relative humidity 40-43%.

The highest indices of survival of β-hemolytic streptococcus were noted during dispersion of bacterial suspensions in a 1% solution of blood serum and a liquid Ringer-Locke's solution with 0.1% gelatin (table 1).

The survival of β-hemolytic streptococcus in an aerosol in relation to humidity was studied at a constant temperature (18-20°) and at various indices of relative humidity (45--92%). In the chamber we sprayed 0.3 ml. (10^9 cells in 1 ml) suspensions of β-hemolytic streptococcus in a Ringer-Locke's solution with 0.1% gelatin. In the conditions of the test conducted, the change of relative humidity of the air in the chamber did not have a significant influence on the survival of streptococci (table 2).

Conclusions

1. An experimental model of a kinetically stable aerosol of β-hemolytic streptococcus was obtained.

2. Blood serum and gelatin had a protective effect on β-hemolytic streptococcus in the experimental aerosol.

3. A low temperature (4°) aided the survival of β-hemolytic streptococcus in the experimental aerosol.

4. Viability of β-hemolytic streptococcus, "protected" by gelatin in Ringer-Locke's liquid, in the bacterial cloud did not depend on the relative humidity of air.

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Table 1

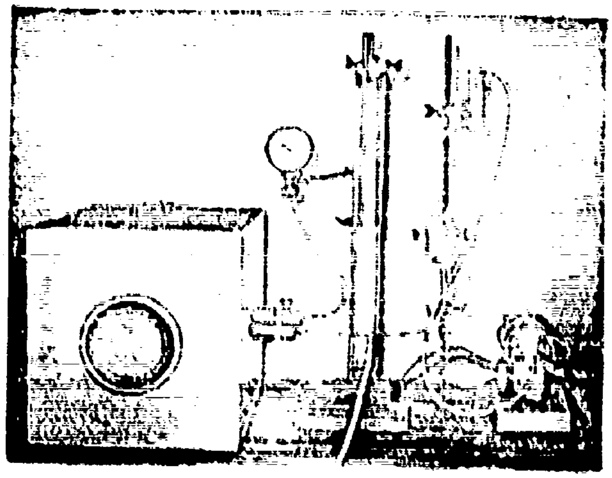
Number of cells of β-hemolytic streptococcus in one liter of aerosol depending on the composition of the dispersed liquid (average indices)

Dispersed liquid	Number of viable cells							Number of tests
	In initial suspension	At the moment of spraying	After					
			15 min.	30 min.	1 hour	$1\frac{1}{2}$ hrs	2 hrs	
Ringer-Locke solution with 0.1% gelatin	$7.94 \cdot 10^7$	$6.48 \cdot 10^5$	$9.16 \cdot 10^4$	$5.55 \cdot 10^3$	498	71	9	5
Physiological solution	$7.26 \cdot 10^7$	$6.8 \cdot 10^5$	$3.68 \cdot 10^3$	274	62	4	-	4
Meat-peptone broth	$7.58 \cdot 10^7$	$6.63 \cdot 10^5$	$7.8 \cdot 10^3$	413	44	18	2.4	5
1% solution of blood serum	$7.34 \cdot 10^7$	$6.99 \cdot 10^5$	$9.08 \cdot 10^4$	$2.3 \cdot 10^3$	$1.5 \cdot 10^3$	222	150	7

Table 2

Number of viable cells of β-hemolytic streptococci in one milliliter of aerosol at various indices of temperature and relative humidity

Indices		Number of tests	Number of viable cells in different periods (in minutes) after spraying				
Relative humidity (in %)	Temperature		5	15	30	45	60
45		12	2 737	54	10	8	2
60		10	2 456	540	77	36	47
75		10	2 200	852	112	20	3
92		15	1 397	265	32	24	4
	+21°	10	2 230	860	118	23	4
	+4°	4	7 524	5 237	3 546	2 220	1 160



Aerosol Chamber